CILIARY AND MUSCULAR LOCOMOTION IN THE GASTROPOD GENUS POLINICES.

MANTON COPELAND,

Searles Biological Laboratory, Bowdoin College.

Introduction.

Although gastropod locomotion has often been made a subject of investigation by naturalists and physiologists with the result that the characteristic muscular form of progression is now fairly well understood, it appears that little is known concerning the peculiar and equally interesting activities of cilia which occur on the feet of numerous species of snails, and the part they may play in locomotion, notwithstanding the fact that Quatrefages as early as 1843 believed them to be locomotor in function. A study of pedal cilia in two species of marine gastropods belonging to the genus *Alectrion* led me to conclude not only that locomotion was due to ciliary action, but also that this action, unlike that of ordinary cilia, was either directly or indirectly under the control of the nervous system (Copeland, '19). Recently similar studies on the genus *Polinices* have been carried on at the Marine Biological Laboratory at Woods Hole, the results of which are here recorded. This group appears to contain the largest animals which have been reported as exhibiting ciliary locomotion. The snails also have a muscular form of progression, thus showing a combination of locomotor methods which I believe has not been described for marine gastropods.

Most of the work has been done on *Polinices* (*Neverita*) *duplicata* (Say), although the more northern species *Polinices* (*Euspira*) *heros* (Say), which is closely similar to the preceding, has been studied to some extent. Unless otherwise specified, observations here noted refer to the former species.

The large foot of *Polinices* is differentiated anteriorly into a well-developed propodium which is prolonged over the head region and a part of the shell. This organ, bluntly pointed at the anterior end, is capable of great extension and freedom of movement, and when protruded bears a striking resemblance to the mammalian

tongue. The remaining portion of the foot is elliptical in outline and possesses a dorsal area which is reflected over a section of the shell. The ventral pedal surface is covered by ciliated epithelium which is richly supplied with mucus. The dorsal surface of the propodium is also ciliated. The effective strokes of the cilia are directed toward the posterior end of the foot and there is no indication that they are ever reversed.

Ciliary Behavior and Locomotion.

When *Polinices* is placed in a glass dish filled with sea water, it usually exhibits a gliding type of locomotion quite distinct from another form of progression which it sometimes employs involving marked muscular contractions of the foot. The former method may be considered first. A close inspection of the foot as it slides over the substrate fails to reveal any indication of rhythmic pedal waves, all regions of the organ moving at the same speed over a thick layer of mucus. Slight muscular contractions produce a rippling motion along the anterior border of the propodium. The same mode of progression is seen when the snail is moving over a sandy or pebbly substrate, and is essentially like that described for *Alectrion obsoleta* (Parker, '11; Copeland, '19) and *Alectrion trivittata* (Copeland, '19), where locomotion has been shown to be due to cilia beating in mucus which covers the sole of the foot and adheres to the substrate (Copeland, '19). A young *Polinices heros* was also noted moving in a similar manner in an inverted position along a band of mucus laid down on the surface film of the water.

In *Alectrion* it was found that actively beating cilia are characteristic of a moving snail, whereas they are quiescent, or nearly so, when the animal is motionless, as would be expected if the cilia function as locomotor organs. Accordingly *Polinices* was first studied with the object of determining whether the behavior of the pedal cilia was like that recorded for *Alectrion*. An adult snail is too large for successful microscopic examination, but a young individual (*P. heros*) whose foot measured 22 mm. in length was observed while moving over a slide in a small amount of water and the cilia on the thin posterior portion of the foot could be seen beating actively. The same animal was again examined when resting with a partly contracted foot in a culture slide, and at this time

all the cilia discernible were either quiescent or showed but slight activity over local areas. The behavior of the pedal cilia of an adult *Polinices* is unquestionably like that of the immature, for when the progression of one is suddenly prevented by holding back the shell, the mucus on the bottom of the foot is driven posteriorly by the beating cilia and objects such as sand grain, pieces of filter paper or even a pin are swept along with it. If now the snail is removed from the water, the propodium may be extended and moved about, at which time the mucus glides over the entire ventral pedal surface and the flicker of beating cilia can be plainly seen by the use of a lens. On the other hand, if the animal is quiescent, either in the water or when held out of it, there is no movement of mucus and in the latter position no indication of ciliary activity is distinguishable.

Polinices placed upside down in a dish of sea water shows a righting reaction which is characteristic. The tongue-like propodium is extended, and the tip directed downward and backward until the ventral anterior margin of the foot is applied to the glass. The attached surface of the propodium is then advanced until the inverted shell lies over the right anterior border of the foot. If the animal does not succeed in applying more of the surface of the foot to the glass, the shell and posterior part of the foot move backward as the propodium progresses, the whole body tending to circle clockwise. If during this activity sand grains are sprinkled over the foot, they are carried posteriorly with the mucus by the beating cilia. No muscular waves are to be seen on the portion of the propodium which is adherent to the glass and locomotion at this time is evidently accomplished by the cilia. On failing to right itself the snail rests with the propodium still in contact with the substrate, or more frequently with the organ withdrawn and held in a somewhat contracted condition with its ventral surface upward. Sand grains now remain on the foot where they are placed and there is no movement of mucus. When, however, the righting activity is resumed the sand and mucus again move down the foot. The righting of the body is finally accomplished after more of the pedal surface is applied to the substrate, particularly that portion along the right side posterior to the propodium, when the rest of the foot and the shell are thrown over to the left. The expanded

hind part of the foot shows no muscular movement during the entire reaction.

The preceding observations lead to the conclusion that pedal cilia are beating actively at the time of locomotion, when the foot is functioning as a righting organ, and when it is being moved about as the snail is held out of the water, and that cilia are quiescent, or nearly so, when the animal is at rest either in the water or out of it. The results of certain experiments may now be recorded which in my opinion leave little doubt that these cilia function as organs of progression.

A comparison of the rate of locomotion with the speed at which objects were driven over the pedal surface by beating cilia, or what may be termed the ciliary rate, was obtained in the following way. A snail which was moving around the periphery of a shallow glass dish filled with sea water was stopped by holding back the shell, but grains of sand adhering to the mucus were carried along the under surface of the foot by the active cilia as the animal attempted to continue its course. By the use of a millimeter rule and mirror placed beneath the dish it was possible with a stop-watch to obtain records of the rate of their movement. One of the lateral margins of the foot was slightly curled upward and some of the records were taken as the sand passed over this area; other grains were timed as they moved with the mucus nearer the central and posterior portions of the expanded foot. The average rate of movement over a distance of 5 mm. in ten trials was found to be 2.7 seconds. Immediately after the ciliary rate was obtained the snail was allowed to proceed over the bottom of the dish and ten records of its speed were secured, which showed that it was moving at the average rate of 5 mm. in 2.6 seconds. The remarkably close correspondence between the locomotor and ciliary rates strongly supports the view that progression is accomplished by ciliary action.

Watching *Polinices* glide over the bottom of an aquarium one gains the impression that the animal is too heavy to be propelled merely by the lashing of cilia in a film of mucus spread over the substrate. That such a conclusion is in reality a false one was proved by a few simple tests. As has been pointed out, the pedal cilia are active when the snail is attempting to right itself, and at that time it is possible to determine their driving power by placing

objects on the expanded ciliary surface. In this manner it was found that a five-cent piece and good-sized pebbles were readily moved by the beating cilia. Following these preliminary tests, a carrier weighing slightly over half a gram was made by fastening a glass ring on a 22 mm. cover glass. A 20-gram weight was then set on the ring, and the cover glass placed on the upturned middle portion of the foot, which in turn was supported so that the cilia were at about the level of the surface of the water. When the snail began the righting reaction the beating cilia moved the mucus and carrier with its load toward the posterior end of the foot. One of the animals tested in this way weighed between 8 and 9 grams in water and had a pedal ciliated surface with foot expanded of about 54 square cm. Thus between 4 and 5 square cm. of active cilia were able to move not less than 20 grams, whereas during locomotion the task of moving 8 grams was distributed over an area of 50 square cm. The largest specimen of *Polinices duplicata* which I was able to obtain weighed only 36 grams in water and its locomotor surface measured about 132 square cm. Although the resistance of the water to be overcome by the moving animal is a factor to be considered in any detailed calculation of the work required of the locomotor apparatus, the results of these tests leave no doubt as to its adequacy.

Although *Polinices* was once observed to move a short distance clinging to the under side of a glass plate with the propodium unattached to the glass, it is the anterior border of this organ which as a rule holds most tenaciously to the substrate when one attempts to dislodge the snail. Several times I have seen one move up the glass side of an aquarium until it came to the surface of the water, when it turned the propodium downward, or perhaps parallel to the surface, and lifted it from the glass. Soon after relinquishing this anterior hold it slipped to the bottom. Moreover, it is able to cling to the under side of a glass plate by the propodium alone and to advance several centimeters by muscular action. Evidently, therefore, this specialized portion of the foot has considerable power of suction, although adhesion by means of mucus is in all probability the principal mode of attachment for the foot as a whole. When the snail is moving the anterior margin of the propodium is in close contact with the substrate and exhibits con-

tinuous though slight muscular contractions. It was conceivable, therefore, that it played some important part in effecting the locomotion of the animal which had been overlooked. In order to test this possibility the anterior portion of a snail's foot was removed so that the locomotor function became restricted to the mid and hind regions. Less than three days after the operation the snail was moving about the aquarium so much like other individuals that it could not be distinguished from them without examining its foot. When moving rapidly, ten records of its speed were taken which showed an average locomotor rate of 5 mm. in 3 seconds. Five records obtained at other times when it was most active averaged 2.6 seconds for the same distance. Another, normal animal, whose speed of locomotion was noted before the preceding tests were made, was moving at the rate of 5 mm. in 3.2 seconds (average of ten trials). After completing this experiment more of the anterior region of the foot was cut away so that there was nothing left which came in contact with the substrate which could possibly be regarded as a portion of the propodium. Again locomotion was not interfered with, and the snail glided both slowly and rapidly over the bottom of the aquarium. It is clear, therefore, that the propodium is not a part of the pedal mechanism which is essential for locomotion.

Muscular Locomotion.

In sharp contrast to the ciliary type of locomotion in *Polinices* is one involving the formation of rhythmic pedal waves. This is a muscular form of progression which begins by the posterior end of the foot being drawn forward initiating a dark transverse band or wave, which travels over the ventral surface of the foot until it reaches the anterior portion when the propodium is thrust ahead. At about the time the propodium is fully extended the hind end of the foot is again contracted and a second wave is produced. This muscular action causes a slight humping of the body, so that the shell rises after the posterior contraction and falls again as the wave passes forward. If the animal is moving very rapidly the anterior end of the foot may be lifted well above the substrate when the wave reaches it, extended and brought down again with considerable force. Although the wave may not be apparent along

the lateral margins of the foot, it does at times extend to this region when, under favorable conditions for observation, it is perfectly clear that the wave is a band-like area of the foot which is temporarily raised above the substrate and moved forward. This interpretation of the pedal wave was offered by Parker in 1911, and later (1917) was shown by him to be true in the case of *Aplysia*. Olmsted ('17) also came to the same conclusion from his studies of Bermudian gastropods.

The speed of muscular locomotion was compared with that dependent on ciliary action alone. A snail whose foot measured about 10.5 cm. in length by 5.7 cm. in width was moving over the bottom of a lead-lined tank in which the water was over 3 cm. deep. By taking five records and averaging them, it was determined that five complete waves passed over the foot in 27.6 seconds, during which time the snail moved forward 7.8 cm. Thus a wave appeared on the average every 5.5 seconds and advanced the foot approximately 1.5 cm. It was moving, therefore, at the rate of one centimeter in about 3.5 seconds. A larger individual with a foot about 15.5 cm. long and 10.7 cm. wide moved much faster. When the results of five trials were averaged it was found that the snail was travelling at the rate of 18.5 cm. in 31.6 seconds, or stated as in the preceding case, a wave started every 6.3 seconds and moved the foot ahead about 3.7 cm. Accordingly the rate of locomotion was approximately a centimeter in 1.7 seconds. Comparing these locomotor rates with those determined for several snails moving without rhythmic contractions, it is evident that the muscular form of progression is faster. For example, the smaller, more slowly moving individual of the two noted above was timed as it glided by ciliary action over the glass bottom of a dish of sea water, a favorable substrate for this type of movement. Twenty records of its speed over distances of one centimeter were taken. The fastest noted was 5 seconds, whereas the series averaged 5.7 seconds.

Undoubtedly pedal cilia are beating when waves are passing over the foot, for they unquestionably are when the snail is burrowing into the sand and the same sort of contraction is in progress. The muscular form of locomotion may pass into the purely ciliary one by the gradual reduction of rhythmic pedal movements

until they entirely disappear. Ciliary progression was observed more frequently than muscular in the case of snails confined in aquaria. The latter type now and then appeared when the animals were moving in a lead-lined tank, over a glass surface and often when they were climbing up the sides of glass aquaria. Ciliary locomotion was successfully accomplished over pebbly bottoms as well as sandy ones. In the former situation the foot, especially the propodium, molded its surface to the irregularities of the substrate and appeared as if it were flowing over them. The remarkable effectiveness of this form of progression is readily understood, however, if it is remembered that irrespective of the character of the substrate the cilia are always beating against a thick layer of mucus which adheres to the underlying materials, and forms a smooth bed over which the foot can readily glide.

If, when moving over a pebbly bottom, the propodium is thrust beneath the stones, the foot is worked forward by muscular contractions, the anterior end in contact with the glass bottom of the aquarium. Thus when considerable resistance is to be overcome the muscular form of progression is employed. This fact is well illustrated by the behavior of a snail burrowing into the sand. Rhythmic contractions go on as in ordinary locomotion. The propodium is directed downward, and when the pedal wave reaches the organ it is extended and worked farther forward and downward into the sand. By holding back a burrowing animal, sand grains may be seen travelling along the sole of the foot and also over the dorsal surface of the propodium, showing that the cilia are beating. By this procedure a snail may completely disappear beneath the sand in less than a minute and a half to a little over two minutes. When the anterior end of the foot was cut off, *Polinices* could not burrow successfully. By continued pedal contractions it was able to work into the sand somewhat, but at the end of about an hour it rested with a part of the shell still exposed. The propodium, therefore, although not necessary in locomotion, is essential for burrowing.

Ciliary Control.

It is now well established that the transmission which excites activity over ciliated epithelia is not ordinarily directed through

nerves, but through the protoplasm of the ciliated cells, a kind of transmission which on account of its resemblance to the nervous type has been designated neuroid. In the course of investigations on the behavior of cilia on the excised foot of the snail *Alectrion trivittata* (Say) the wave-like spread of action was clearly observed (Copeland, '19). Cilia quiescent over a limited area of the foot suddenly became active, others close by then began beating, and as the wave of action continued many were in motion. After a time they came to rest again. To account for this behavior, and the fact that cilia start beating over the entire ventral surface of the foot when the snail begins locomotion, one might assume that resting cilia are excited to movement in some definite region of the foot through impulses received from the nervous system, and that neuroid, or intraepithelial transmission, spreading from this center, brings about the activity of the remaining cilia. This possibility is rendered improbable, however, from the fact that if two observers are watching carefully the sand-covered surface of the foot of *Polinices* when the snail begins the righting reaction, no difference can be detected in the time when the sand grains begin movement, although the two points under observation may be ten centimeters apart.

In order to gain more definite information on the method of transmission in *Polinices* the following experiments were tried. After anesthetizing a snail in magnesium sulphate, the epithelium near the center of the foot was cut in such a way that an area 25 mm. long and 18 mm. wide was isolated from the surrounding epithelium. When the animal became active again after the operation the cilia on the isolated epithelium behaved the same as those over other parts of the foot, as far as could be determined by testing with sand grains. As long as the snail rested upside down there was no movement of the mucus or sand on the foot, but when it attempted to right itself ciliary activity began on the isolated patch of epithelium, as well as elsewhere, and continued until the animal rested again. Following this test more of the epithelium was cut away so that the subepithelial tissues were exposed in a well-defined band completely surrounding the epithelial island. The ciliary behavior was the same as before. The test was repeated on *Polinices heros,* an area of epithelium 13 to 15 mm. in

diameter being separated from the surrounding portions near the center of the foot. Again the ciliary action over the isolated part was coördinated with that of the rest of the pedal surface. These experiments prove that impulses exciting ciliary action may be transmitted elsewhere than through epithelial cells, and point to a subepithelial or nervous pathway. Other tests on two examples of *Polinices heros* substantiated this view. The tissue lying beneath the epithelium was severed in a plane parallel with the surface. The cuts extended inward from near the border of the foot posterior to the propodium, leaving most of the overlying epithelium in contact with that of the rest of the foot, but isolating it from the deeper portions of the organ over an area, in one case, about 15 mm. long by 4 to 7 mm. wide, and in the second instance, approximately 8 mm. by 13 mm. Many tests were made during righting reactions, and although sand grains moved with the mucus directly in front and back of the areas cut under, and also to one side of them, they remained quiescent over the isolated regions themselves. The operation caused the epithelium to become somewhat contracted, but numerous tests on normal individuals have shown that a wrinkling of the pedal surface does not in any mechanical way inhibit the beating of cilia.

Conclusions.

The view that gastropod locomotion is not always a muscular process is well supported by the behavior of *Polinices* where two types of progression occur in a single species, one of which is immediately recognized as muscular, whereas the other presents entirely different features and upon analysis appears to be dependent on ciliary action.

The general ciliary behavior in *Polinices,* and especially the results of the experiments just described, uphold strongly the conclusions already drawn from a study of two species of the genus *Alectrion,* namely, that the control of ciliary action has been taken over by the nervous system, perhaps directly, or conceivably through connections with muscle or mucous cells associated with the ciliated cells. Intraepithelial transmission may play some part in effecting the spread of ciliary action, but evidently impulses

directed over deeper pathways are the primary ones concerned in regulating this movement.

LITERATURE CITED.

Copeland, M.

'19 Locomotion in Two Species of the Gastropod Genus *Alectrion*, with Observations on the Behavior of Pedal Cilia. BIOL. BULL., Vol. 37, pp. 126–138.

Olmsted, J. M. D.

'17 Notes on the Locomotion of Certain Bermudian Mollusks. Jour. Exp. Zoöl., Vol. 24, pp. 223–236.

Parker, G. H.

'11 The Mechanism of Locomotion in Gastropods. Jour. Morph., Vol. 22, pp. 155–170.

Parker, G. H.

'17 The Pedal Locomotion of the Sea-Hare *Aplysia californica*. Jour. Exp. Zoöl., Vol. 24, pp. 139–145.

de Quatrefages, A.

'43 Mémoire sur l'Éolidine paradoxale (*Eolidina paradoxum* Nob.). Ann. Sciences Naturelles, Sér. 2, Tome 19, pp. 274–312.